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(54) **ANALYTICAL APPARATUS**

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APPAREIL D'ANALYSE

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(73) Proprietor: **The Secretary of State for Defence
Farnborough, Hampshire GU14 0LX (GB)**

(72) Inventor: **PERKINS, Elaine, Ann
Wiltshire SP4 0JQ (GB)**

(74) Representative: **Bowdery, Anthony Oliver
D/IPR (DERA) Formalities,
Poplar 2,
MOD Abbey Wood#19
Bristol BS34 8JH (GB)**

(56) References cited:
WO-A-90/05295
US-A- 5 437 840
US-A- 5 485 277

- **PETERLINZ K A ET AL: "TWO-COLOR APPROACH FOR DETERMINATION OF THICKNESS AND DIELECTRIC CONSTATN OF THIN FILMS USING SURFACE PLASMON RESONANCE SPECTROSCOPY" OPTICS COMMUNICATIONS, vol. 130, no. 4/06, 1 October 1996, pages 260-266, XP000627753**

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Description

TECHNICAL FIELD

[0001] The present invention relates broadly to apparatus for the detection of analytes. The invention further relates to methods employing such apparatus.

BACKGROUND

[0002] The use of Surface Plasmon Resonance (SPR) for the detection of small soluble analytes from solution is well known (see e.g. "Advances in Biosensors - A Research Annual Vol 1. 1991" Ed. A P F Turner, Pub. Jai Press Ltd, London).

[0003] Briefly, an SPR apparatus generally comprises a light source for generating polarised light; a sensor, the outside of which is metal coated and may be contacted with a sample solution, and means for detecting the light which is internally reflected from the inner sensor surface.

[0004] In the absence of bound analyte, light is totally internally reflected at an incident angle characteristic of the refractive index (RI) of the sensor and of the sample solution. At a particular incident angle (the 'SPR angle'), interaction of the metal with the evanescent wave set up by internal reflection of the polarised light causes a drop in intensity of the reflected light. This drop can be observed using the light detector.

[0005] The binding of analyte to the sensor surface, within the evanescent wave zone, alters the RI of the sensor and this perturbs the SPR angle. This perturbation can be observed using the light sensor and related to the surface concentration of analyte.

[0006] SPR detection in the literature has generally been limited to use with soluble molecular size analytes e.g. biomolecules such as proteins and nucleic acids which are specifically bound within the evanescent zone using appropriate ligands.

[0007] US-A-5485277, and the paper "Two-colour approach for determination of thickness and dielectric constant of thin films using surface plasmon resonance spectroscopy" in Optics Communications, Vol. 130, No. 4/06 1 October 1996, pages 260-266, by Peterlinz et al describe various types of surface plasmon resonance (SPR) apparatus. Patent US-A-5437840 describes an apparatus which determines the amount of an analyte by detecting changes in refractive index or resonant frequency changes.

[0008] However, these apparatus and techniques have not been suitable for accurately detecting sample materials with both soluble and insoluble analytes therein. In particular, due to the more limited way in which (for instance) roughly spherical cell of several pm diameter interact with the evanescent zone, only fairly high concentrations (e.g. 107-108 ml) have been detected using SPR. Thus in order to detect cells, as opposed to (for instance) protein antigens, further apparatus, and hence more cost, time and experimentation, have been required. For instance cells have frequently been detected-using culture techniques followed by specific detection.

[0009] WO-A-90 05295 also discloses an SPR apparatus and further suggests the measurement of light scattered by analyte. The collected light intensity is used as a measure of total analyte present.

[0010] According to the present invention, a surface plasmon resonance apparatus for detecting a soluble or particulate analyte, comprises the features set out in claim 1.

[0011] Preferably the first detector being located on the opposite side of the sensor surface from that on which light from the source generating the evanescent wave is incident. Suitable sensors are slides

[0012] Possible analytes may include those particulate or insoluble analytes containing or consisting of biomolecultes, for instance bacteria or other cells, spores, viruses or virions etc., or biomolecules themselves such as proteins or polynucleotides. Possible bacterial targets include cryptosporidium, E. coli, salmonella etc.

[0013] The apparatus may thus be used with a wide variety of samples suspected or known to contain analytes. For example environmental samples such as water, or biological samples.

[0014] Broadly speaking the apparatus operates as follows: in use the second detector detects the binding of soluble analytes to the sensor surface by detecting the changes in the intensity of light internally reflected from the sensor surface, whereas the first detector detects particulate analytes bound to the sensor surface by detecting the light scattered therefrom. The apparatus of the present invention is therefore capable of the sensitive detection of both soluble and particulate analytes, and thus may provide a quicker, cheaper or more sensitive alternative to the methods and apparatus presently used in the art.

[0015] It is important to stress the different functions of the detectors in the apparatus. The second detector must be arranged to detect light internally reflected from the sensor surface, the intensity of this light being dependent on the SPR effects occurring as analytes (especially soluble ones) bind at the sensor surface altering the refractive index of the sensor/sample interface. The detector may be a 2-D array detector as described in more detail in the Examples below.

[0016] By contrast the first detector detects light which is scattered by analytes (especially particulate ones) which interact with the evanescent field at the sensor surface. This may give a sensitivity for detecting large particulate analytes several orders of magnitude higher than would be obtainable using pure SPR. Clearly the nature of the first detector used will determine the sensitivity and acuity of the detection, but in preferred embodiments single cells bound within the evanescence zone may be detected and resolved using the first detector while the bulk binding effects of soluble molecules

may be detected using the second.

[0017] In one embodiment the first detector is located on the same side of the surface as the light source such as to be capable of detecting light which is back-scattered when an analyte is bound to thereto.

[0018] The term 'light source' as used herein means any source of light radiation, including where appropriate the tip of an optical fibre which is attached to a remote radiation source.

[0019] In a different embodiment, the first detector is located on the opposite side of the surface as the light source detector such as to be capable of detecting light which is scattered when an analyte is bound to thereto.

[0020] In either case it may be desirable that the first detector is located such as to be capable of detecting light scattered at a predetermined angle, for example substantially normally, to the sensor surface. This will minimise interference from light which is being totally internally reflected from the surface.

[0021] Generally the sensor block will comprise a prism or a hemicylinder, such as are 'known to those skilled in the art of SPR detection. The sensor block is adapted to receive the detachable sensor which provides the metallised surface. The adaptation may simply consist of providing a general area to mount the sensor such as a slide, or the block may be specially shaped or configured to receive it e.g. in a groove or properly-dimensioned well.

[0022] The block and or sensor may in addition be adapted to form all or part of one wall of a flow channel, through which a liquid sample can flow in liquid contact with the metallised surface. An apparatus comprising such a flow channel forms one embodiment of the first aspect of the invention.

[0023] Preferably the metallised sensor surface is adapted or otherwise funetionalised such as to facilitate the immobilisation of macromolecules which are capable of specifically binding biomolecules thereto. For instance the sensor may have a hydrophilic dextran surface. Antibodies may then be immobilised thereto in order to specifically bind antigenic analytes. Alternatively a polynucleotide probe may be immobilised for specifically binding a polynucleotide analytes.

[0024] Preferably the e.g. antibodies are bound only to discrete portions of surface in order to facilitate the detecting light which is scattered when an analyte is bound to thereto.

[0025] These portions are then visualised (and possibly further resolved) by the first detector as contrasting discrete bright areas against the darker portions of the surface which do not have marcomolecules bound to them.

[0026] The surface may have greater than one type of macromolecule immobilised thereto for specifically binding greater then one type of antigen. The different types of e.g. antibody may be bound in known discrete areas in order to easily identify which antigen is being specifically bound.

[0027] In one further embodiment of the invention, the apparatus includes a second light source. This can be used to increase the intensity of the light scattered from the sensor surface when an analyte is bound thereto. Although this embodiment requires additional components, it has the advantage that the light source can be optimised (e.g. wavelength, angle of incidence against the sensor surface, intensity) for light scattering.

[0028] It may be desirable to locate the second light source such as to minimise the amount of stray light emitted therefrom which is detected by the second detector.

[0029] This may be done by locating the second light source such that light emitted thereform travels along the same light path but in the opposite direction from the light from the first light source which is internally reflected from the sensor surface to the second detector, as is shown in the Figures below.

[0030] The light source(s) used can be selected without undue burden by those skilled in the art. In order to maximise intensity, and hence sensitivity, the or each light source may be a laser light source, or a light emitting diode.

[0031] In a second aspect of the invention, a method of detecting a soluble or particulate analyte comprises the steps set out in claim 8.

[0032] For instance a soluble analyte in a sample may be detected by detecting the changes in the intensity of light internally reflected from the sensor surface. A particulate analyte in a sample may be detected by detecting the light scattered from the analytes bound to the sensor surface. Preferably the apparatus is arranged such that soluble or particulate analytes may be detected simultaneously.

[0033] The means adapted to secure the first detector may comprise a holder or clamp positioned and/or dimensioned to receive the camera and associated optics, such that it can detect light scattered from the sensor surface. The holder or clamp may be moveable in a pre-determined way to facilitate the function of the first detector when in place e.g. to allow focusing.

[0034] Preferably the means are adapted to secure the first detector such that it is capable of detecting light scattered at a predetermined angle, for example substantially normally, to the sensor surface.

[0035] The second detector of the apparatus may also be adapted such as to receive a second light source. The adaptation may be such that the second light source, when in place, is configured to minimise interference with the second detector by being directed away from it, as described above.

FIGURES

[0036]

Fig 1 Shows a schematic diagram of a surface plasmon resonance apparatus for detecting a soluble or

a particulate analyte, as described in more detail in Example 1.

Fig 2 Shows a block diagram of the complete instrument of Example 1.

Fig 3 Shows how the apparatus may be used to detect multiple analytes. Fig 3(a) and (b) show the light source, hemicylinder (plus detection surface), and CCD array detector schematically. Fig 3(c) shows a detail of the CCD array.

Fig 4 Shows bound particles scattering light from the metallised detection surface of a hemicylinder sensor. The light can be detected by a video camera (not shown).

Fig 5 Shows scattering from bacterial particles above a silver surface: the points of light represent scattered light from *Erwinia herbicola*.

EXAMPLES

EXAMPLE 1: SURFACE PLASMON RESONANCE APPARATUS FOR DETECTING A SOLUBLE OR A PARTICULATE ANALYTE

[0037] Fig 1 Shows a schematic diagram of a surface plasmon resonance apparatus for detecting a soluble or a particulate analyte, such as could be constructed (in the light of the present disclosure) by those skilled in the art. A block diagram of the components of the apparatus is shown in Fig 2.

[0038] This system may be rearranged if desired, for instance the polariser may be placed after the hemicylinder if required.

[0039] Considering Fig 1, the light path to the second detector ('CCD Array') is from the light source at the left, through the beam splitter (which splits a portion to the reference detector), through a polariser and focusing lens, off the internal surface of the hemicylinder, through a collimating lens and into the CCD array.

[0040] The light path is shown schematically in Fig 3 (a). An extended collimated source may be used to illuminate the hemicylinder surface continuously over a range of incident angles, as shown in Fig 3(b). The CCD array is composed of a pixelated array of individual light sensors, each detecting a different reflected angle or being used to detect a different sample analyte (in this case 4 different samples) as shown in Fig 3(c). This allows the rapid monitoring without moving parts.

[0041] Considering Fig 1, the light path to the first detector ('CCD camera') is from the light source at the left, through the beam splitter (which splits a portion to the reference detector), through a polariser and focusing lens and onto the hemicylinder.

[0042] The intensity is supplemented in this embodiment by light from the visible laser diode on the right which travels away from the CCD array and through the collimating lens on the right and onto the hemicylinder. The evanescent field generated on the upper, metallised, surface of the hemicylinder causes particles bound therein to scatter light as depicted in Fig 4. The scattered light is focused through a lens and detected by the CCD camera.

[0043] Devices according to Example 1 may be constructed based on existing SPR machines but having the additional components described above. The machines and components may be those available commercially. For instance the light source may advantageously be an edge emitting LED as used in fibre-optic communications (e.g. EG&G type S86018). A stabilised power supply may be used to minimise artefacts.

[0044] The sensor may be metal-coated microscope slide (or similar thickness dielectric) which is index matched onto the hemicylinder with fluid of similar refractive index. A portion of the hemicylinder may be ground off to accommodate the slide.

[0045] The CCD array (with 'pixels' about 20 μm^2) may be of a type developed for video use. Readout from CCD was accomplished by transferring a sample-area row to a readout or row register. Correlated Double Sampling (CDS) may be used to eliminate noise. The analog output can be passed to a digital signal processor via an ADC. A suitable processor is an Analog Device ADSP-2105. This can communicate with an external host PC via a bi-directional parallel port.

[0046] The CCD video camera can be a conventional, commercially available, one e.g. as sold by Hamamatsu (Japan).

EXAMPLE 2: METHOD OF USE OF SURFACE PLASMON RESONANCE APPARATUS

[0047] In use, in order to correct for differences in source intensity along the collimated bean, a calibration can be carried out before the experiment. The sensor surface is then exposed to the sample(s). The host selects monitoring angles through using reflectivity vs. angle scans. Data is then acquired over a set time period and displayed by the host PC.

EXAMPLE 3: DETECTION OF PARTICULATE ANALYTE USING THE SECOND DETECTOR

[0048] In order to illustrate the light scattering technique, a glass microscope slide was coated with silver for optimum surface plasmon resonance (48nm). The slide was then mounted onto a glass hemicylindrical prism and illuminated with a 3mW helium-neon laser. The slide was covered with a film of bacteria (*Erwinia herbicola)* at $1x10^6$/ml in phosphate buffered saline solution. The bacteria were then allowed to adsorb onto the surface of the silver microscope slide.

[0049] The output from the CCD array above the SPR surface is a normal video output with 256 levels of bright-

ness. Observation above the silver surface showed that initially all pixels on the CCD camera gave a low reading (1-20) and the surface appeared dark. As the bacteria approached the surface, the brightness increased for those pixels specifically aligned with the areas where the bacteria were on the surface. The maximum brightness level recorded from the light scattered by the bacteria at the surface was 230. The appearance of the surface was that of a dark background with bright spots associated with the bacteria on the surface (See Figure 5).

[0050] As a control, a film of phosphate buffered saline without bacteria was used to cover the silver surface of a similar microscope slide. This time, no scattering from the surface was observed.

Claims

1. A surface plasmon resonance apparatus for detecting soluble or particulate analyte, the apparatus comprising:

   a sensor providing a metallised surface for binding the analyte;

   a light source arranged to generate an evanescent wave at the sensor surface;

   a first detector arranged to detect light scattered by analyte particles bound to the metalised surface and

   a second detector arranged to detect light from the evanescent wave which is internally reflected from the metalised surface:

   characterised by said first detector being a CCD camera arranged to permit visualisation of the distribution of analyte over the sensor surface from the light scattered thereby.

2. An apparatus as claimed in claim 1 wherein said first detector is located on the opposite side of the sensor surface from that on which light from the source generating the evanescent wave is incident.

3. An apparatus as claimed in claim 1 wherein the first detector includes optical focusing means.

4. An apparatus as claimed in any preceding claim wherein the first detector is arranged to detect light scattered at a predetermined angle to the sensor surface.

5. An apparatus as claimed in any preceding claim and further **characterised by** a second light source for increasing the intensity of light scattered from an analyte bound to the sensor surface.

6. An apparatus as claimed in claim 5 and further **characterised in that** the second light source is arranged to transmit light along the same light path, but in the opposite direction, to the light from the evanescent light source which is internally reflected from the sensor surface to the second detector.

7. An apparatus as claimed in claim 5 or 6 wherein the second light source transmits light of a different wavelength from that of the evanescent light source.

8. A method of detecting a soluble or particulate analyte comprising:

   (a) binding the analyte to a metallised surface comprised in a sensor;

   (b) generating an evanescent wave at the sensor surface;

   (c) detecting light scattered by analyte particles bound to the metallised surface using a first detector and

   (d) detecting light from the evanescent wave which is internally reflected from the metallised surface using a second detector,

   characterised by said first detector being a CCD camera arranged to permit visualisation of the distribution of analyte over the sensor surface from the light scattered thereby.

9. A method as claimed in claim 8 wherein the analyte is selected from the list comprising: a prokaryotic cell; a eukaryotic cell; a virus or virion proteins and a nucleic acid.

Patentansprüche

1. Oberflächenplasmonresonanzvorrichtung zur Erfassung einer löslichen oder partikelförmigen zu analysierenden Substanz mit einem Sensor mit einer metallisierten Oberfläche zum Binden der zu analysierten Substanz,
   einer Lichtquelle zur Erzeugung einer abklingenden Welle auf der Sensoroberfläche,
   einem ersten Detektor zur Erfassung von durch auf der metallisierten Oberfläche gebundene, zu analysierende Partikel gestreutem Licht und
   einem zweiten Detektor zur Erfassung des Lichts von der abklingenden Welle, das von der metallisierten Oberfläche intern reflektiert wird;
   dadurch gekennzeichnet, daß der erste Detektor eine CCD-Kamera ist, die eine Visualisierung der Verteilung der zu analysierenden Substanz auf der

Sensoroberfläche anhand des durch sie gestreuten Lichts ermöglicht.

2. Vorrichtung nach Anspruch 1, bei der der erste Detektor auf der der Seite, auf die das Licht von der die abklingende Welle erzeugenden Quelle auftrifft, gegenüberliegenden Seite der Sensoroberfläche angeordnet ist.

3. Vorrichtung nach Anspruch 1, bei der der erste Detektor eine optische Fokussiereinrichtung umfaßt.

4. Vorrichtung nach einem der vorhergehenden Ansprüche, bei der der erste Detektor gestreutes Licht in einem vorgegebenen Winkel zur Sensoroberfläche erfaßt.

5. Vorrichtung nach einem der vorhergehenden Ansprüche, weiter **gekennzeichnet durch** eine zweite Lichtquelle zur Steigerung der Intensität des **durch** eine auf der Sensoroberfläche gebundene, zu analysierende Substanz gestreuten Lichts.

6. Vorrichtung nach Anspruch 5, weiterhin **dadurch gekennzeichnet, daß** die zweite Lichtquelle Licht entlang des gleichen Lichtpfads, jedoch in der in bezug auf das Licht von der abklingenden Lichtquelle, das von der Sensoroberfläche intern zum zweiten Detektor reflektiert wird, umgekehrten Richtung abstrahlt.

7. Vorrichtung nach Anspruch 5 oder 6, bei der die zweite Lichtquelle Licht mit einer anderen Wellenlänge als die abklingende Lichtquelle abgibt.

8. Verfahren zur Erfassung einer löslichen oder partikelförmigen, zu analysierenden Substanz mit den Schritten:

   (a) Binden der zu analysierenden Substanz auf einer in einem Sensor enthaltenen, metallisierten Oberfläche,

   (b) Erzeugen einer abklingenden Welle auf der Sensoroberfläche,

   (c) Erfassen des von den auf der metallisierten Oberfläche gebundenen, zu analysierenden Partikeln gestreuten Lichts unter Verwendung eines ersten Detektors und

   (d) Erfassen des von der metallisierten Oberfläche intern reflektierten Lichts der abklingenden Welle unter Verwendung eines zweiten Detektors,

   dadurch gekennzeichnet, daß der erste Detektor eine CCD-Kamera ist, die eine Visualisierung der Verteilung der zu analysierenden Substanz auf der Sensoroberfläche anhand des von ihr gestreuten Lichts ermöglicht.

9. Verfahren nach Anspruch 8, bei dem die zu analysierende Substanz aus einer Liste ausgewählt wird, die prokaryotische Zellen, eukaryotische Zellen, Viren oder Virenpartikel, Proteine und Nukleinsäuren umfaßt.

Revendications

1. Appareil à résonance au plasmon de surface adapté pour détecter un analyte soluble ou parriculaire, l'appareil comprenant :

   un détecteur pourvu d'une surface aimantée adaptée pour provoquer l'adhésion de l'analyte ;
   une source lumineuse configurée de manière à produire une onde évanescente au niveau de la surface du détecteur ;
   un premier détecteur configuré pour détecter la lumière diffusée par les particules de l'analyte collé à la surface aimantée ; et
   un deuxième détecteur configuré pour détecter une lumière en provenance de l'onde évanescente qui est réfléchie en interne à partir de la surface aimantée :

   caractérisé en ce que ledit premier détecteur est une caméra DTC configurée pour permettre de visualiser la répartition de l'analyte sur la surface du détecteur par l'intermédiaire de la lumière diffusée sur celle-ci.

2. Appareil selon la revendication 1, dans lequel ledit premier détecteur est placé du côté opposé de la surface du détecteur à partir de laquelle la lumière provenant de la source qui produit l'onde évanescente est incidente.

3. Appareil selon la revendication 1 dans lequel le premier détecteur comprend des moyens de mise au point optique.

4. Appareil selon l'une quelconque des revendications précédentes, dans lequel le premier détecteur est configuré de manière à détecter la lumière diffusée à un angle prédéterminé par rapport à la surface du détecteur.

5. Appareil selon l'une quelconque des revendications précédentes et **caractérisé en outre par** une deuxième source lumineuse adaptée pour augmenter l'intensité de la lumière diffusée à partir de l'analyte collé à la surface du détecteur.

6. Appareil selon la revendication 5 et **caractérisé en outre en ce que** la deuxième source lumineuse est configurée de manière à transmettre la lumière le long de la même trajectoire de lumière, mais dans la direction opposée, par rapport à la lumière provenant de la source lumineuse évanescente qui est réfléchie en interne à partir de la surface du détecteur jusqu'au deuxième détecteur.

7. Appareil selon la revendication 5 ou 6, dans lequel la deuxième source lumineuse transmet une lumière d'une longueur d'onde différente par rapport à celle de la source lumineuse évanescente.

8. Procédé de détection d'un analyte soluble ou particulaire, comprenant les étapes consistant à :

(a) faire adhérer l'arialyte à une surface aimantée comprise dans un détecteur ;
(b) produire une onde évanescente à la surface du détecteur ;
(c) détecter la lumière diffusée par les particules d'analyte collées sur la surface aimantée en utilisant un premier détecteur ; et
(d) détecter la lumière en provenance de l'onde évanescente qui est réfléchie en interne à partir de la surface aimantée, en utilisant un deuxième détecteur,

caractérisé en ce que ledit premier détecteur est une caméra DTC configurée pour permettre de visualiser la répartition de l'analyte sur la surface du détecteur par l'intermédiaire de la lumière diffusée sur celle-ci.

9. Procédé selon la revendication 8 dans lequel l'analyte est choisi à partir de la liste comprenant : une cellule procaryote ; une cellule eucaryote ; un virus ou des protéines de virion et un acide nucléique.

Fig.1.

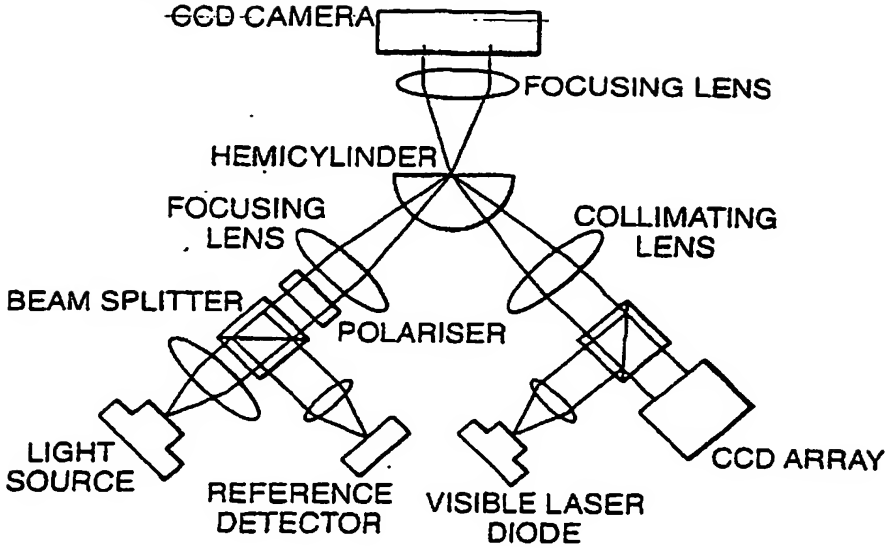
CCD CAMERA
FOCUSING LENS
HEMICYLINDER
FOCUSING LENS
COLLIMATING LENS
BEAM SPLITTER
POLARISER
LIGHT SOURCE
REFERENCE DETECTOR
VISIBLE LASER DIODE
CCD ARRAY

Fig.2.

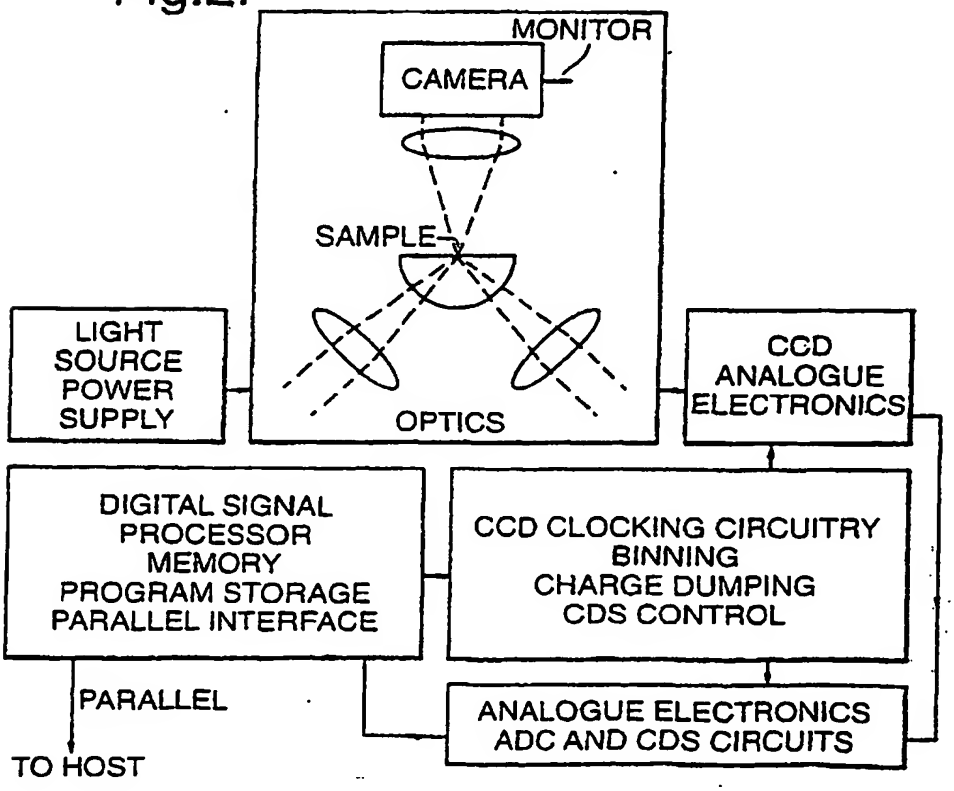
MONITOR
CAMERA
SAMPLE
LIGHT SOURCE POWER SUPPLY
OPTICS
CCD ANALOGUE ELECTRONICS
DIGITAL SIGNAL PROCESSOR MEMORY PROGRAM STORAGE PARALLEL INTERFACE
CCD CLOCKING CIRCUITRY BINNING CHARGE DUMPING CDS CONTROL
PARALLEL
TO HOST
ANALOGUE ELECTRONICS ADC AND CDS CIRCUITS

Fig.3(a).

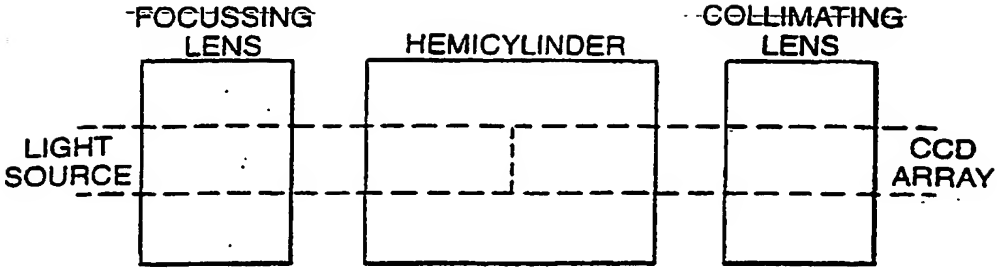

Fig.3(b).

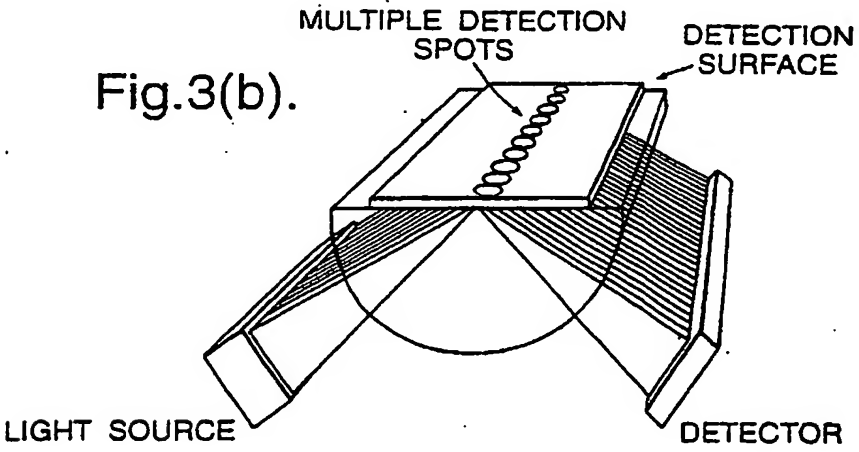

Fig.3(c).

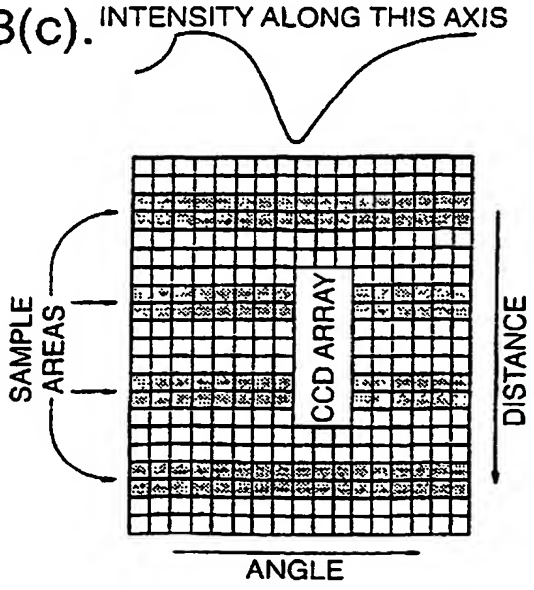

Fig.4.

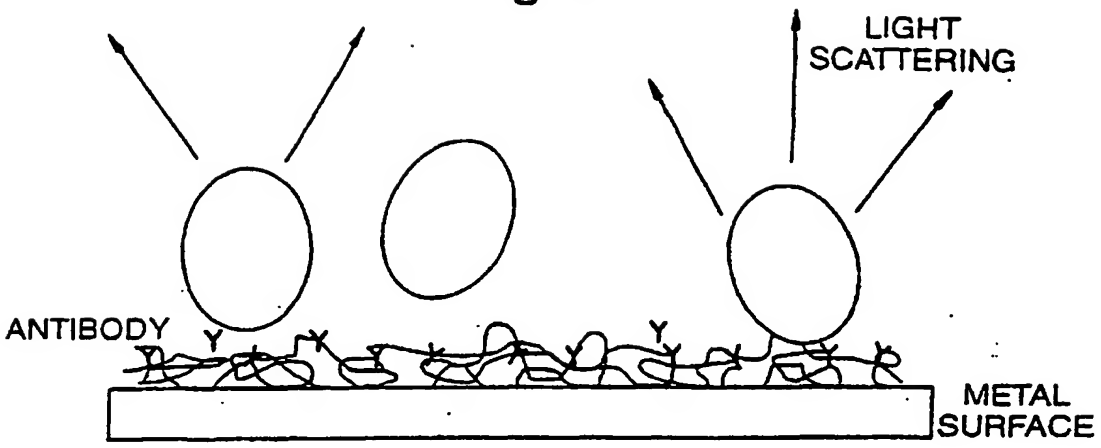

Fig.5.